### **California Environmental Protection Agency**

### **Air Resources Board**

### DETERMINATION OF ALDEHYDE AND KETONE COMPOUNDS IN AUTOMOTIVE SOURCE SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Standard Operating Procedure No. 104 Revision 2.1

June 1999

Organic Analysis Section Southern Laboratory Branch Monitoring & Laboratory Division 9528 Telstar Avenue El Monte, CA 91731

This report has been reviewed by the staff of the California Air Resources Board and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Air Resources Board nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

#### 1 Introduction

- 1.1 This procedure describes a method of sampling and analyzing automotive engine exhaust for aldehyde and ketone compounds (carbonyls) in the range of 0.02 to 200 micrograms (µg) in 2,4-dinitrophenylhydrazine (DNPH) impregnated cartridges. Currently the target compounds analyzed and reported by this method are: formaldehyde, acetaldehyde, acrolein, acetone, propionaldehyde, butyraldehyde, hexanal, benzaldehyde, methyl ethyl ketone (MEK), methacrolein, crotonaldehyde, valeraldehyde, and m-tolualdehyde.
- 1.2 This procedure is derived from a method used by the U.S.EPA (Ref. 9.1).

#### 2 Method Summary

- 2.1 Sample Collection:
- 2.1.1 For routine motor vehicle testing, the vehicle is tested according to the Federal Test Procedure (FTP, Ref. 9.2), using a dynamometer (dyno) and constant volume sampler (CVS) to dilute the exhaust for sampling.
- 2.1.2 Samples are also received from CVS testing using non-FTP driving cycles, Sealed Housing Evaporative Determinations (SHEDs, Ref. 9.2), cartridge samples for round-robin testing and carbonyl-containing samples from other miscellaneous sources.
- 2.1.3 The automotive test personnel collect the carbonyl samples by flowing dilute exhaust (approximately 1.0 liter/min. flow rate) through cartridges (Ref. 9.3). The samples are then brought to the laboratory for analysis.
- 2.2 Elution and Analysis:
- 2.2.1 Each cartridge contains an absorbing compound (2,4-DNPH) which complexes with the carbonyl compounds to form their dinitrophenylhydrazone derivatives. The cartridges are then eluted with 5.0 milliliters (mL) acetonitrile and analyzed (Ref 9.4).
- 2.2.2 Separation and analysis is performed using a High Performance Liquid Chromatograph (HPLC) with an ultraviolet (UV/VIS) detector.

#### 3 Interferences and Limitations

3.1 An interferant is any detectable compound present in the sample with a retention time similar to that of any target carbonyl described in this method. To reduce interference error, proof of chemical identity may require periodic confirmations using an alternate

method and/or instrumentation, e.g., alternative HPLC columns or mobile phase

compositions.

- 3.2 If samples are not analyzed the same day as received, they must be refrigerated at a temperature below 40° F. Refrigerated samples are stable for up to 30 days.
- 3.3 MEK and butyraldehyde coelute when using the primary system (Section 4.1.6.1); therefore, the secondary system is used to separate them (Section 4.1.6.2).
- 3.4 The elution of cartridges with 5.0 mL acetonitrile performed in the laboratory established a statistical average eluted volume of 4.4 mL; the remaining 0.6 mL is retained in the cartridge.

#### 4 Instruments and Apparatus

- 4.1 Waters model HPLC analytical system consists of the following:
- 4.1.1 Dual high pressure pumps (Waters model 510)
- 4.1.2 Pump module controller
- 4.1.3 Liquid autosampler (Waters model 717 WISP)
- 4.1.4 Sample tray cooler set at 20° C (Waters WISP cooling model)
- 4.1.5 Temperature control module (Waters)
- 4.1.6 Chromatographic Columns
  - 4.1.6.1 <u>Primary system:</u> two Supelcosil columns [Supelco, Inc., 4.6 millimeters (mm) inside diameter (ID) x 25 centimeters (cm) long] in series and a guard column [2.0 cm long packed with LC-18, 5 micrometer (μm) pellicular beads]
  - 4.1.6.2 <u>Secondary system:</u> one Delta Bond AK column (Keystone Scientific, Inc.,
    4.6 mm ID x 200 mm) and a guard column (4.6 mm ID x 2.0 cm long packed with LC-18, 5 μm pellicular beads).
- 4.1.7 UV/VIS Detector (Waters model 486 Tunable Absorbance Detector)
- 4.1.8 PC-based data system for peak integration (Millennium model 32)

#### 5 Reagents and Materials

5.1 For sample collection: DNPH impregnated cartridges (SEP-PAK DNPH Silica cartridges from Waters)

- 5.2 Acetonitrile (ACN), HPLC grade, Burdick and Jackson or equivalent
- 5.3 Purified water, HPLC grade, Burdick and Jackson or equivalent
- 5.4 Methanol, HPLC grade, Burdick and Jackson or equivalent
- 5.5 Stock solution Aldehyde/Ketone-DNPH Standard Solution-13, by Radian Corporation, consisting of thirteen compounds (see Table 1), each having a concentration of 3.0 μg/mL and 99% purity. The concentration is the amount of carbonyl compound (NOT the carbonyl-DNPH derivative) per mL of solution.
- 5.6 <u>Working Standard</u> A working standard is prepared as needed by diluting 2.0 mL of the stock solution (3.0  $\mu$ g/mL) to 10 mL (v/v) using ACN for dilution. The working standard concentration is 0.6  $\mu$ g/mL.
- 5.7 <u>Control Standard</u> A quality control standard, containing all target carbonyl/2,4-DNPH derivatives within the typical concentration range of real samples, is analyzed to monitor the precision of the analysis of each target carbonyl. The control standard is prepared by batch mixing old samples and stirring for a minimum of two hours. The solution may be spiked with the 3.0  $\mu$ g/mL or 15  $\mu$ g/mL stock solutions or individual compound standards, if needed, to obtain the desired concentration levels of the target analytes. All target compounds except acrolein have been found to be stable in the control standard.

DNPH Derivative	Chemical Formula	Molecular Weight (g/mole)	Melting Point (°C)	
Formaldehyde	C <sub>7</sub> H <sub>6</sub> N <sub>4</sub> O <sub>4</sub>	210.15	165-166	
Acetaldehyde	C <sub>8</sub> H <sub>8</sub> N <sub>4</sub> O <sub>4</sub>	224.18	152-153	
Acetone	C <sub>9</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub>	238.20	125-127	
Acrolein	C <sub>9</sub> H <sub>8</sub> N <sub>4</sub> O <sub>4</sub>	236.19	165 (dec)	
Propionaldehyde	C <sub>9</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub>	238.20	144-145	
Crotonaldehyde	C <sub>10</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub>	250.21	185-188	
MEK (2-Butanone)	C <sub>10</sub> H <sub>12</sub> N <sub>4</sub> O <sub>4</sub>	252.23	117-118	
Methacrolein	C <sub>10</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub>	250.21	200-201	
n-Butyraldehyde	C <sub>10</sub> H <sub>12</sub> N <sub>4</sub> O <sub>4</sub>	252.23	119-120	
Benzaldehyde	C <sub>13</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub>	286.25	240-242	
Valeraldehyde	C <sub>11</sub> H <sub>14</sub> N <sub>4</sub> O <sub>4</sub>	266.26	107-108	
m-Tolualdehyde	C <sub>14</sub> H <sub>12</sub> N <sub>4</sub> O <sub>4</sub>	300.27	212	
Hexanal	C <sub>12</sub> H <sub>16</sub> N <sub>4</sub> O <sub>4</sub>	280.28	106-107	

#### 6 Procedure

- 6.1 DNPH-impregnated cartridges are used to collect carbonyl samples from automotive exhaust. Separate background samples are collected during each of the three test modes.
- 6.2 Each cartridge is extracted by connecting it to a syringe assembly and eluting with 5.0 mL of acetonitrile. The liquid containing the carbonyl-DNPH derivatives is allowed to flow by gravity into a glass storage container until all of the yellow color band has been eluted. The plunger is then pressed to expel the excess ACN from the cartridge. Approximately 0.6 mL of ACN is retained in the cartridge; no carbonyls are retained in the cartridge.
- 6.3 Approximately 0.75 mL of the eluent is then transferred into a 1.0 mL autosampler glass vial and sealed with a plastic snap cap.

- 6.4 The sample vials are loaded onto the autosampler for injection in the following order:
- 6.4.1 Working standard, control standards, ACN blank, field blank, and samples.
- 6.4.1 A stock standard (3.0  $\mu$ g/mL) is loaded at the end of the sequence if there is a sample that exceeds the working standard concentration (0.6  $\mu$ g/mL).
- 6.5 The HPLC standard operating conditions are:

#### 6.5.1 Primary System

Columns	Analytical column - Supelcosil, 4.6 mm ID x 25 cm long, two columns in series  Guard column - 4.6 mm ID x 2.0 cm long packed with LC-18,  5 μm pellicular beads		
Column oven temperature	40° C		
Detector	UV/VIS at 360 nanometers (nm)		
Sample volume	10 μL		
Solvent A	ACN		
Solvent B	10% (v/v) methanol in water		
Flow	1.05 mL/minute (min)		
Program	50% A, 50% B; 0 (initial time)		
	65% A, 35% B; 0 to 2 min (gradient)		
	100% A, 0% B; 2 to 22 min (gradient)		
	50% A, 50% B; 22 to 37 min (gradient)		
Data system	PC-controlled data acquisition system		

MEK and butyraldehyde tend to coelute in the primary system. The standard's chromatogram is shown in Figure 1 with peak # 8 as "MEK + butyraldehyde". To separate these compounds, it is necessary to analyze the samples using a secondary system.

#### 6.5.2 Secondary System

Columns	Analytical column - Delta Bond AK (4.6 mm ID x 200 mm long) packed column Guard column - 4.6 mm ID x 2 cm long packed with LC-18,  µm pellicular beads (The guard column is replaced every 5 to 6 analysis days to prolong the life of the analytical column.)
Column oven temperature	40°C
Detector	UV/VIS at 360 nm
Sample volume	10 μL
Solvent A	ACN
Solvent B	ACN in water, 35 % (v/v)
Flow	1.5 mL/min
Program	0 % A, 100% B; 0 (initial time)
	23% A, 77% B; 0 to 5.5 min
	46% A, 54% B; 5.5 to 18 min
	0% A, 100% B; 18 to 30 min
Data system	PC controlled data acquisition system

This secondary system is not used to report all compounds because formaldehyde tends to coelute with a non-carbonyl compound. MEK is reported by this system. The standard chromatogram is shown in Figure 2 with peak # 7 as MEK. Butyraldehyde concentration is calculated according to Section 7.4.

- 6.6 The peaks are identified and quantified by the data system. All chromatograms are checked for proper identification and baseline drift. Any misidentification and drifting in the baseline are manually corrected by the reintegration of the chromatograms.
- Only the target carbonyl peaks at or above the maximum allowable detection unit (LOD) are reported.

#### 7 Calculations

- 7.1 For each target compound the carbonyl mass is calculated from its carbonyl/2,4-DNPH mass.
- 7.2 The mass of each carbonyl compound per cartridge, is determined using the following calculation:

Mass  $_{sample}$  (µg) = Peak Area  $_{sample}$  x RF x Cartridge Elution Volume (mL)

where

$$RF = \frac{Concentration_{standard} (\mu g/mL)}{Peak_{standard}}$$

and Cartridge Elution Volume is defined as 4.4 mL (Section 3.4)

- 7.3 For tolualdehyde, the sum of all isomers present (m-, p- and o-) is reported as m-tolualdehyde.
- 7.4 Under the conditions of both primary and secondary systems in section 6.6, butyraldehyde is calculated using the following formula:

$$[Butyraldehyde] = [Butyraldehyde + MEK]_{Supelcosil} - [MEK]_{Delta Bond}$$

#### **8** Quality Control

- 8.1 Reagent Blank The solvents used are of the highest HPLC grade and are tested for impurities when a new lot number is used. If this lot is found to be acceptable (no carbonyl compounds present at concentrations at or above the LOD), daily blank analysis is not required.
- 8.2 <u>DNPH Carbonyl Purity</u> The carbonyl/2,4-DNPH solutions are checked and certified for purity by Radian Corporation by their chromatograms and melting points. Analysis of the solution of carbonyl/2,4-DNPH solution must yield only the peaks of interest. No contaminant peaks above the LOD should be observed.
- 8.3 <u>Calibration Run</u> One run of the calibration standard is required daily to generate the response factors (RF) needed for quantifying sample analysis. Three injections of the calibration standard are recommended to ensure the instruments are stabilized; only the last injection is used to generate the RF.

- 8.4 Control Standard Run One run of the quality control standard is required daily after the calibration run (see Figure 3). More runs of the quality control standard are recommended at a rate of ~10 % of the samples analyzed. Measurements of all compounds in the control standard, except acrolein, must fall within the control limits before sample analysis may proceed. To meet this requirement it may be necessary to rerun the calibration and/or control standard, and/or inspect and repair the HPLC.
- 8.5 <u>Control Charts</u> A quality control chart is maintained for each component of the control sample, except acrolein. The control charts, used on a daily basis, establish that the method is in control. The following statements describe how to construct a typical control chart:
  - (1) obtain at least 20 daily standard results;
  - (2) calculate the control standard mean concentration and standard deviation(s) for the target analyte; and
  - (3) create a control chart for the target analyte by placing the concentration on the Y-axis and the date on the X-axis. Establish upper and lower warning limits at two standard deviations  $(2\sigma)$  above and below the average concentration, and control limits at  $(3\sigma)$  above and below the average concentration.

The concentrations of all target analytes contained in the control standard must be within the control limits ( $3\sigma$  above or below the mean) for the sample results to be acceptable. Due to the low variability of the carbonyl control standard measurement, a control standard measurement which exceeds the  $3\sigma$  limit, but is less than 10 % above or below the mean, is also considered to be in control. A measurement is considered to be out of control when the measured value exceeds both the  $3\sigma$  and 10 % limits, or if all measurements on two consecutive days exceed the  $2\sigma$  limit. Because of its instability, no control requirement has been established for acrolein.

- 8.6 <u>Field Blank</u> One field blank cartridge is analyzed daily, from the same lot number as the sample cartridges. If cartridges from two different lot numbers are used for sampling on a given day, blank cartridges from both lots must be analyzed.
- 8.7 <u>Duplicates</u> A duplicate analysis of a sample is performed at least once per day. The average of the two duplicates is reported. The relative percent difference (RPD) is calculated for each duplicate run as follows:

RPD = <u>Difference between duplicate & original measurements x 100</u>
Average of duplicate & original measurements

For each compound, the allowable RPD depends on the average concentration level for the duplicate runs, as shown in Table 2.

Table 2

Average Measurement	for Duplicate Runs	Allowable RPD (%)		
1 to 10	Times LOD	100		
10 to 20	· · · · · · · · · · · · · · · · · · ·	30		
20 to 50	٠٠ ,,	20		
Greater than 50	66 27	15		

If the results of the duplicate analyses do not meet these criteria for all target carbonyls, the sample must be reanalyzed. If the criteria are still not met, all samples analyzed during the day from this instrument must be reanalyzed. An example of a replicate QC chart is given in Figure 4.

- 8.8 <u>Linearity</u> A multipoint calibration to confirm instrument linearity is performed for all target analytes for new instruments, after making instrument modifications which can affect linearity, and at least once every six months. The multipoint calibration consists of at least five concentration levels, each above the LOD and distributed over the range of expected sample concentration. Each concentration level is measured at least twice. A linear regression analysis is performed using concentration and average area counts to determine the regression correlation coefficient (r). The (r) must be greater than 0.995 to be considered sufficiently linear for one-point calibrations. An example of a multipoint calibration determination is shown in Figure 5.
- 8.9 <u>Limit of Detection</u> The LOD for the target analytes must be determined for new instruments, after making instrument modifications which can affect the LOD and at least once every six months. To make the calculations, it is necessary to perform a multipoint calibration consisting of at least four low concentration levels, each above the expected LOD. The lowest standard's concentration should be between one to five times the estimated LOD. The two lowest concentration standard is run a minimum of six times while the others are run a minimum of four times. The LOD is calculated using the following equation:

$$LOD = |b| + (t \times \sigma)$$

where

|b| = is the absolute value of the y-intercept;

m = is the slope of the linear regression;

 $\sigma$  = is the standard deviation of at least 6 replicate determinations of the lowest concentration standard; and

t = is the t-factor for 99 percent confidence for a one-sided normal (Gaussian) distribution. (See Table 3.)

Table 3

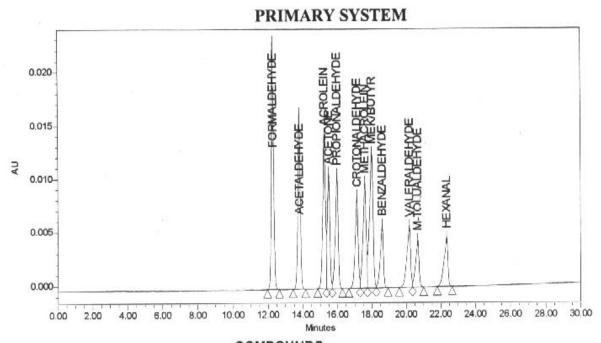
Degrees of Freedom (n-1)	t-value
4	3.7
5	3.4
6	3.1
7	3.0

An example of an LOD determination is shown in Figure 5.

- 8.9.1 The maximum allowable LOD is 0.06 µg carbonyl derivative/mL. The calculated laboratory LOD must be equal to or lower than the maximum allowable LOD. All peaks identified as target compounds that are equal to or exceed the maximum allowable LOD must be reported. If the calculated laboratory LOD is less than the maximum allowable LOD, the Southern Laboratory Branch (SLB) may choose to set its reporting limit at the maximum allowable LOD, the calculated laboratory LOD or any level in between.
- 8.9.2 For the purpose of calculating the total mass of all species, the concentrations of the compounds below the LOD are considered to be zero.
- 8.10 Quality control of the sampling procedure is overseen by the clients.

#### 9 **REFERENCES**

- 9.1 Hull, L.A., "Procedures for 2,4 Dinitrophenylhydrazone Aldehyde Ketone Air Analysis", Internal Report of the U.S. EPA.
- 9.2 Code of Federal Regulations (CFR), Title 40, Part 86.
- 9.3 Tejada, S.B. "DNPH-Coated Silica Cartridges for Sampling Carbonyl Compounds in Air and Analysis by HPLC", EPA/MSERB internal report, 1985 and Tejada, S.B., "Evaluation of Silica Gel Cartridges Coated in Situ with Acidified 2,4-DNPH for sampling Aldehydes and Ketones in Air", Inter. J. Environ. Anal. Chem., 1986, Vol. 26, pp. 167-185.
- 9.4 Air Resources Board, "California Non-methane Organic Gas Test Procedures", Part F.



3	Name	Retention Time	Amount	Area	Height
1	FORMALDEHYDE	12.277	0.600	209062	23109
2	ACETALDEHYDE	13.811	0.600	156854	16445
3	ACROLEIN	15.256	0.600	155262	14472
4	ACETONE	15.512	0.600	109600	10784
5	PROPIONALDEHYDE	15.992	0.600	122771	10667
6	CROTONALDEHYDE	17.149	0.600	101248	8640
7	METHACROLEIN	17.611	0.600	127009	9878
8	MEK/BUTYR	18.012	1.200	184112	12676
9	BENZALDEHYDE	18.611	0.600	72396	5858
10	VALERALDEHYDE	20.193	0.600	86255	5744
11	M-TOLUALDEHYDE	20.680	0.600	59514	4419
12	HEXANAL	22.351	0.600	72285	4572

Figure 1

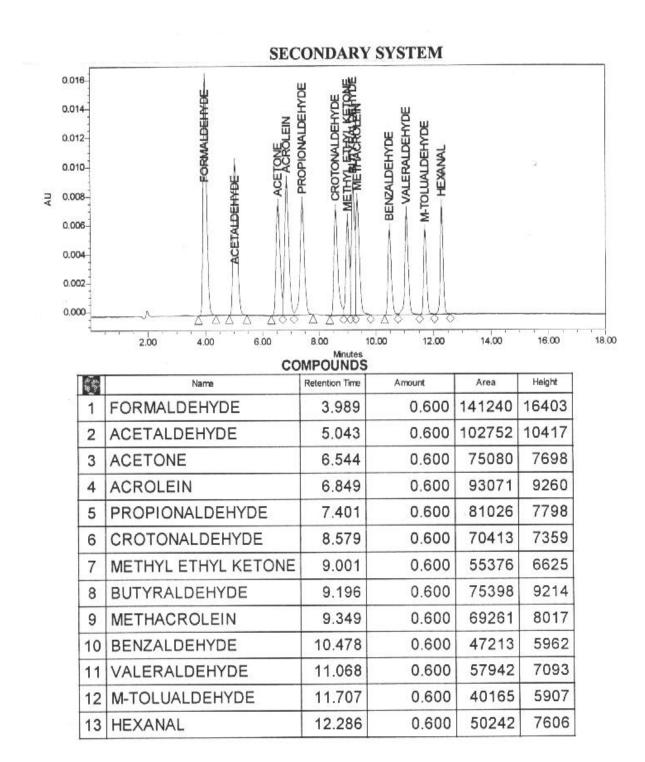
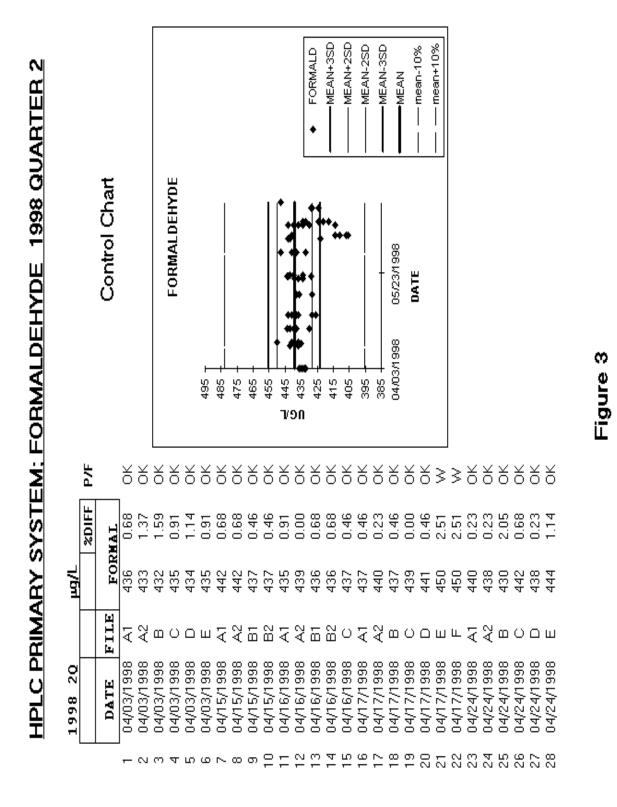


Figure 2



			Formaldehyde				
				(Pi	rimary Sys	tem)	
				LOD=	9.00		
			RUN	RUN		MAX.	
	DATE	IEST #	#1	#2	%RPD	%RPD	<b>STATUS</b>
1	04/03/1998	10R5	92	93	1	30	OK
2	04/03/1998	1C2	375	376	0	20	ok
3	04/03/1998	14R5	84	84	0	100	OK
4	04/03/1998	11C4	152	153	1	30	OK
5	04/03/1998	11C5	136	137	1	30	OK
6	04/03/1998	3C13	114	115	1	30	OK
7	04/03/1998	906	52	52	0	100	OK
8	04/03/1998	907	55	55	0	100	OK
9	04/03/1998	10C2	63	63	0	100	OK
10	04/15/1998	13C2	212	212	0	20	OK
11	04/15/1998	13C3	203	202	0	20	OK
12	04/15/1998	3C14	137	137	0	30	OK
13	04/15/1998	2C8	93	93	0	30	OK
14	04/17/1998	3015	126	126	0	30	OK
15	04/17/1998	13C4	226	228	1	20	OK
16	04/17/1998	8C10	61	60	2	100	OK
17	04/17/1998	1C9	46	46	0	100	OK
18	04/17/1998	3C16	222	220	1	20	OK
19	04/17/1998	69UC1	347	345	1	20	OK
20	04/17/1998	10C3	82	82	0	100	OK
21	04/17/1998	1C7	67	68	1	100	OK
22	04/17/1998	13C1	176	176	0	30	OK
23	04/24/1998	8C11	71	71	0	100	OK
24	04/24/1998	1C11	64	63	2	100	OK
25	04/24/1998	14C1	176	176	0	30	OK
26	04/24/1998	9C8	44	43	2	100	OK
27	04/24/1998	9C9	39	40	3	100	OK
28	04/24/1998	76C1	743	742	0	15	OK
29	04/24/1998	78C1	806	822	2	15	OK
30	04/24/1998	83C1	3691	3731	1	15	OK

Figure 4

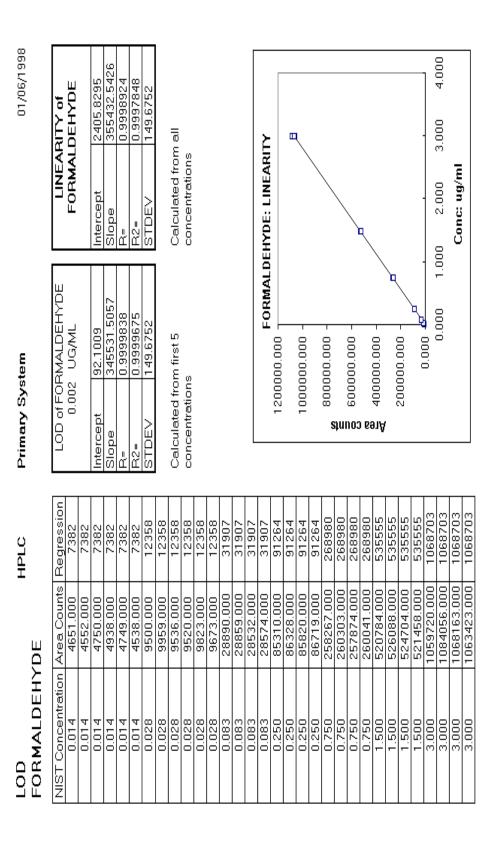


Figure 5